GIARDIA AND THE WATERBORNE TRANSMISSION OF GIARDIASIS

A GENERAL REVIEW

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WSDG Report
WSDG-TP-00003
September 1981

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ABSTRACT

A general review of the organism Giardia sp. is presented to familiarize wildland hydrologists with the organism, its impact upon humans and its occurrence in the natural environment. Also discussed are current efforts to sample for Giardia sp., anticipated data needs and the potential role of the U.S.D.A. Forest Service in Giardia sp. studies.

An adaptation to the wildland setting of the existing U.S. Environmental Protection Agency (EPA) sampling technique is described. Included are figures describing the organism and a prototype Forest Service Instream Giardia Sampler.

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1.0 Introduction

Increasingly, wildland hydrologists in the Forest Service are asked for information on Giardia and the health hazard it poses. As more attention is focused on this organism through increases in disease occurrence and media coverage, it is likely that in the future additional concern and questions will be generated.

The Forest Service publication FS-359 entitled, "Is the Water Safe? Think Before You Drink", was produced to provide general information on this subject. Its brevity, however, may result in the hydrologist being asked for clarification. So that he may be better able to provide such information, the following review is presented. It is not exhaustive, but should provide a broad base from which the hydrologist may assist both management and the general public. Furthermore, it can serve as a source of information to begin additional data gathering efforts if necessary.

2.0 The Disease

Giardia lamblia, a flagellated protozoan, is the most commonly diagnosed flagellate of the human intestinal tract [1,31,32,33,34,35]. It has been estimated to occur in up to 7.4% of otherwise healthy Americans [32], and in 1% to 20% of the population worldwide [33]. This organism, while endemic to most of the world, has become an item of increasing concern to wildland recreationists, especially to those of the Rocky Mountain area. National attention has been paid this organism since epidemic levels were observed in American travelers returning from Leningrad and Tokyo [2,3]. In addition, epidemic level occurrences in United States resort towns (Aspen, Vail, etc.) [4,5,7,8] and other

municipalities (Berlin, New Hampshire; Camas, Washington; Boulder, Colorado; etc.) [6,9,29,30] have drawn further attention to the organism and its associated disease, Giardiasis.

Although the largest single documented U.S. outbreak occurred in Rome, New York in 1975 [2,16] with other cases documented in California, Oregon, Pennsylvania, Utah, Idaho, Arizona, Kentucky and North Carolina [29,30,36,37,38], the bulk of the noteworthy "outbreaks" has occurred in Colorado. There has also been idenfitied a national increase in incidences (estimated to number 70 between 1965 and 1978) [10], but this may not be a real trend. Some clinicians contend the trend is in diagnosis rather than incidence.

The disease produced by this organism is not generally life threatening. The organism, by means of a sucking disc, attaches itself in the trophozoite or active form to the upper part of the small intestine (duodenal-jejunal mucosa) rarely entering the gall bladder or biliary tree [3]. Clinicians have established that reversible damage occurs in the intestinal mucosa [32,34]; however, there remains some question as to the significance of this effect [39].

In humans, the symptoms of this disease include malodorous diarrhea, flatulence (intestinal gas), anorexia (loss of appetite), malaise (weakness, discomfort), weight loss, nausea, midepigastric cramps, and abdominal distention (bloating) [3]. Investigators have also observed a "malabsorption syndrome" in some giardiasis patients. They have reported that Vitamin A "[11] . . . D-xylose, fat, or vitamin B_{12} . . ." [32] were malabsorbed in approximately 60% of the patients studied [32]. This aspect of the disease is especially pronounced in juveniles and certain ethnic groups [3,11].

The prepatent period (time between infection and diagnosis) varies to some degree but generally averages about nine days [12,13]. Symptoms may last indefinitely [3,12], and observers have commonly reported ranges of 5 to 41 days [12] and 2 to 6 weeks [13]. In addition, spontaneous remission of symptoms was reported as a common phenomenon by one observer [12], and acquired immunity has been described by others [39].

Symptomatic individuals tend to fall into one of three classes of symptom severity, i.e., chronic, sub-acute and acute. In the acute stage the symptoms are as described previously and may also include vomiting, chills, low grade fever and headache. These symptoms may last from a few days to a few months. The sub-acute stage is characterized by intermittent episodes of the above but generally not of the same severity. These and less severe symptoms could also last for months [13]. The chronic stage can display fewer symptoms but can also result in the shedding of cysts for prolonged periods.

The asymptomatic is the most common form of infection. The length of time during which such an infected individual may shed cysts has not yet been determined [13], but it is suspected that cysts may be excreted for months or years [31].

The disease is generally successfully treated through drug therapy. Drugs employed include quinacrine (Atabrine) hydrochloride, metronidazole (Flagyl), furazolidone (Furoxone), tinidazole (Flasigyn), acranil, chloroquine, amodiaquin hydrochloride and camoquin [1,2,3,11]. Of these, only the first three drugs are licensed for treatment of Giardiasis in the United States. The successful treatment rate tends to decrease in the order of presentation above. In the U.S., quinacrine is the most widely prescribed of these drugs [31] and Wolfe has reported a cure rate of at least 95% using this drug [39].

Thses drugs are not without side effects. Quinacrine has been associated with dizziness, headache, vomiting, psychotic reactions and blue or yellow staining of the skin. Metronidazole has also been associated with nausea, headache and vomiting. It is also associated with diarrhea and has been reported to be carcinogenic, teratogenic and mutagenic in nonhuman test subjects [31].

In view of the foregoing, physicians must exercise caution in the prescription of the above drugs. Specifically, quinacrine is contraindicated for individuals with psychosis or psoriasis, and all of the above are contraindicated for pregnant individuals [3]. Special care must be exercised in any treatment for individuals suffering from ulcers or similar gastrointestinal disorder (i.e., gall bladder).

It has been observed that patients who have been successfully treated utilizing chemotherapy are subject to reinfection if exposed subsequent to treatment. However, untreated patients and asymptomatic patients tend to maintain a degree of immunity to reinfection [14].

Some question remains as to the mode of transmission of the organism and the dosage required to induce clinical symptoms. It is generally accepted, however, that this organism is waterborne. This was clinically documented for the first time in Rome, New York in 1975 [2,9]. Studies have also determined that Giardia may be cross transmitted from various animal hosts to humans and possibly vice versa [29,30,31,36,40,41]. Early research with prisoner volunteer subjects indicated encapsulated dosages of as little as 10 cysts could be adequate to produce clinical symptoms but left unanswered questions as to minimum dosage and mode of natural transmission [12].

3.0 The Organism

Giardia lamblia was first observed in 1681 by Leeuwenhoek in a microscopic examination of his own stools [32]. Recognizably precise description, however, was not accomplished until 1859 when Lambl gave the organism the name intestinalis. Subsequent confusion over naming was, in part, eliminated by Stiles in 1915 who created the binomial Giardia lamblia in honor of Professor A. Giard of Paris and Doctor F. Lambl of Prague [1]. Stiles' efforts, however, have not entirely eliminated the confusion. This is primarily a consequence of imperfect knowledge of the organism's morphology. As a result, this organism is called Giardia lamblia in the western hemisphere and western Europe, while in the Soviet Union and eastern Europe it is called Lamblia intestinalis, and the disease is called Lambliasis [31].

Early researchers felt there were several species of Giardia and each was highly host specific. Consequently, there arose the species names bovis (cattle), caprae (sheep), canis (dog), cati (cat), duodenalis (rabbit), caviae (guinea pig), muris simoni (Norway rat) and muris microti (house mouse) [15]. Recent cross transmission studies and electron microscopic examination have cast substantial doubt upon this classification schema [16]. There is currently a tendency to identify only two or three species of Giardia [13,14]. However, further study is required.

Giardia lamblia has been phylogenetically classified [1,13] as follows:

Kingdom: Animalia - Linnaeus, 1758

Phylum: Protozoa - Goldfuss, 1818, emend. Von Siebold, 1895

Subphylum: Sarcomastigophora - Honigberg and Balamuth, 1963

Superclass: Mastigophora - Diesing, 1866

Class: Zoomastigophora - Calkins, 1909

Order: Protomonadina

Family: Hexamitidae

Genus: Giardia

Species: Lamblia - Stiles, 1915,

and other species

The organism has a two stage life cycle characterized by cystic and trophic stages. The mature cyst tends to have four nuclei situated at one end of the organism and divides to form two binucleated individuals when excystation occurs. The cyst is ovoid or ellipsoid and measures 8 to 12 µm by 6 to 10 µm (see Figure 1).

The trophozoite is pear-shaped, bilaterally symmetrical and measures 9 to 21 µm in length, 5 to 15 µm in width, and 2 to 4 µm in thickness (see Figures 1, 2 and 3). The trophozoite is further characterized by two nuclei, four pairs of flagella and a notched concavity which occupies virtually all of the anterior-ventral half of the organism [1,13]. This concavity is the sucking disc which attaches to the duodenal-jejunal mucosa. It has been postulated that the ventral flagella serve to create a flow of liquid towards the low pressure area created by the sucking disc through a beating action which facilitates both attachment and nourishment [27].

Reproduction in the trophozoite form is accomplished through longitudinal binary fission resulting in two daughter trophozoites. Encystation is incompletely understood at this time but is apparently triggered when diarrhea diminishes, resulting in dehydration of liquid feces during transit through the colon.

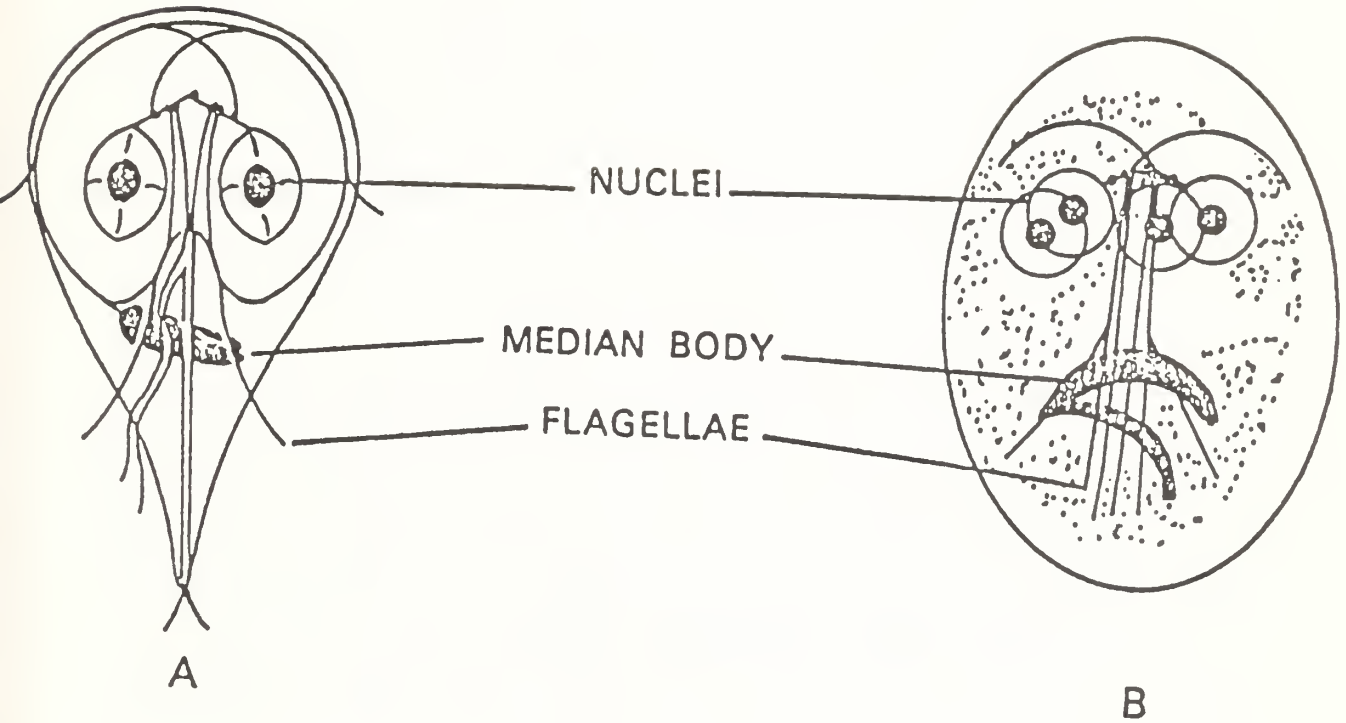

Figure 1. A . Giardia Trophozoite. B. Giardia Cyst.
(not drawn to scale; adapted from Levine [15])

Little information is readily available on the ability of cysts to remain viable under environmental extremes. Cysts have been observed to survive in cold or tepid water for from one to three months, and at temperatures of down to -20°C for up to ten hours. The organism is, however, immediately killed at 50°C [3,15,17].

Recent research indicates that at least one species of Giardia contains endosymbiotic bacteria [1,2]. These bacteria exist within the cytoplasm of the cyst and trophozoite forms and have an incompletely understood relationship to the host. It is felt, however, that they probably serve an adaptive function and may offer potential for control of the disease in humans [42].

In the cyst form, this organism is very resistant to disinfection. Early studies indicated that it could survive 0.5 percent chlorinated water for two to three days [17]. Recent studies have established the doses of various disinfectants necessary to kill cysts, as defined by excystation, at several levels of water temperature, pH and clarity [35]. The concentrations and contact times were mostly greater than those normally encountered in municipal water supply systems.

4.0 Giardia in the Natural Environment

As noted earlier, the first instance in which the waterborne transmission of Giardia was documented occurred in Rome, New York in 1975 [2,9]. Consequent to the above, researchers have attributed the endemic occurrence of this organism in Colorado to the consumption of untreated mountain stream water [18].

The lack of adequate understanding of organism morphology and classification has resulted in some confusion as to the degree and avenues

by which the oganism moves from host to host if, in fact, such transfer occurs. This has resulted in significant cross transmission studies by various researchers [16,21]. One of the more ambitious efforts is being carried out by Dr. Charles P. Hibler and associates at Colorado State University, Wild Animal Disease Center.

Although far from providing definitive answers, encouraging results have been produced. At this time, data principally indicate which animal species are infected by Giardia derived from humans. An assumption of a converse relationship is utilized as a working hypothesis, but remains to be proven [19].

Numerous animal species have been identified as hosts for Giardia; however, the first documented instance in which animal infection was clinically associated with an epidemic level occurrence of Giardiasis in humans was in Camas, Washington in the spring of 1976 [9,20]. On this occasion, 3 out of 12 live-trapped animals were found to be infected with Giardia sp. All infected animals were beavers. Of interest is the following: (1) The contaminated animals were trapped outside the Camas Municipal Watershed, but within foraging distance of the watershed; (2) Giardia sp. cysts were identified in the raw water entering Camas municipal intakes; (3) Although no human contamination was observed on the watershed itself, such was observed in the area in which infected animals were trapped [9].

In Ontario, Canada, Giardia sp. was identified in 98.8 percent of meadow voles (Microtus pennsylvanicus) and 98 percent of deer mice (Peromyscus maniculatus) live-trapped in a 1978 study [21].

At this time, initial results indicate the beaver plays a central role in the transmission of Giardia. Up to 18 percent of beavers studied have

been found to be positive for Giardia sp. cysts in studies in Colorado mountain areas [19]. In addition, such beaver infection has been observed to be related to human activity. Specifically, while beaver populations have been found to be positive for infection in areas of human activity, they have often been found to be negative in areas upstream of such acitivity [14].

The significance of infected individuals, whether human or animal, to the spread of the organism is difficult to assess. A single human subject has been observed to excrete 2.19×10^6 cysts per gram of formed stool [13]. Consequently, a few infected individuals may have substantial impact upon downstream water users.

5.0 Sampling for Giardia

The bulk of Giardia studies have involved the examination of the stools of infected individuals. These examinations have resulted in morphological and cross transmission studies to evaluate infectivity, species differentiation and host specificity. These studies were additionally supported by epidemiology studies and sanitary surveys in instances of known outbreaks. Virtually all of the foregoing methodologies have been indirect in approach.

In the past, the main reason for reliance upon such indirect techniques has been the lack of a suitable in vitro cultivation technique for organism growth from the cyst stage [13]. Lacking such a technique, there has been no truly suitable method by which a determination might be made as to cyst viability and infectivity, outside of experimental infection. Furthermore, without such a technique, cysts isolated from wild animals or environmental waters have revealed little information as to potential human impact.

Recent work by Iger and Gaitonde [43] and Meyer [22,44] in developing an in vitro methodology for cultivating Giardia lamblia offers significant opportunities to better understand the ultrastructure and function of this organism. Some of these opportunities have begun to develop as evidenced by recently revised estimates of the cysticidal effects of disinfectants [35,45,46].

The use of scanning and phased electron microscopy for both surficial and ultrastructure studies of the organism are aiding in the development of a better understanding of both the organism and its internal function (see Figures 2 and 3). This is demonstrated by the recent discovery of endosymbionts in Giardia (i.e., life forms residing within the cytoplasm of Giardia) [42]. This and similar discoveries will provide opportunities for the evaluation and, finally, control of viability and infectivity of Giardia cysts and trophozoites.

Until a procedure is developed to evaluate the presence of Giardia by using indicator organisms or similar techniques, the principal method by which Giardia cysts can be identified from a sample of any kind is through transmission microscopic examination of all materials sampled. Viability and infectivity in the past have largely been inferred through staining techniques (e.g. eosin) [16] or experimental animal infection. This may change with the development of the in vitro techniques mentioned.

Until recently, sampling for Giardia consisted of fecal examinations only. However, to gain an understanding of the waterborne aspect of this disease, the EPA and the Center for Disease Control (CDC) have made efforts to develop techniques to sample raw and treated water to isolate the organism for study.

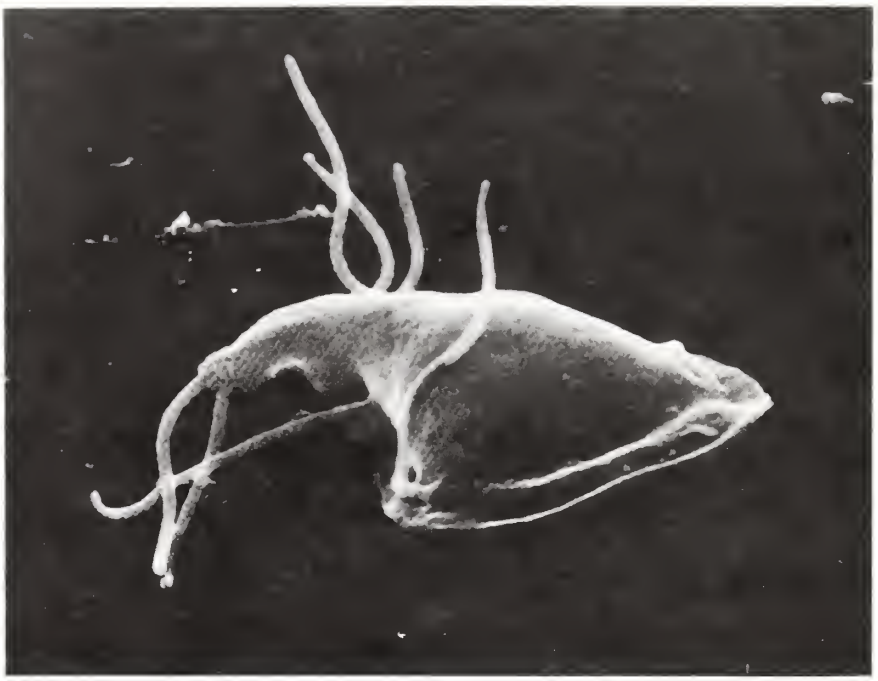

Figure 2. Dorso-lateral view of G. muris trophozoite isolated from small intestine (15,000 x), by Feely and Erlandsen from Meyer and Jarroll, 1980 [31].

Figure 3. Ventral view of G. muris trophozoite isolated from small intestine. Note the prominent ventral adhesive disc (21,500 x), by Freely and Erlandsen from Meyer and Jarroll, 1980 [31].

Initial attempts to isolate Giardia in Aspen, Colorado in 1965 [23] utilized the membrane filter technique [11,29]. This technique proved unsuccessful because of difficulties in sampling large volumes of turbid water.

Following this, the CDC employed a diatomaceous earth, pressure swimming pool filter to examine water during the 1975 Rome, New York outbreak [2,9,13]. This technique examined (filtered) large volumes of water (over 280,000 gallons) in 10 days [8]. The results of this study were not definitive and, although large quantities of water were filtered, the equipment was large and cumbersome and results were not quantitative.

In 1976, the EPA developed a filtration technique which was employed in Camas, Washington and Berlin, New Hampshire [13]. This technique employed an orlon yarn wound filter with a porosity of 7 μm. The filter was incorporated in a plastic holder which was connected to inlet and outlet hoses and a flow meter [13]. The sampler, while in itself being compact and lightweight, required a faucet connection or pump.

In using this procedure, the filter is removed from the collection system after filtering an appropriate amount of water and transported to a laboratory. In the lab the orlon windings are cut from the central core and washed in distilled water. The resultant residue is then fixed with Formalin, stained with Lugol's iodine and vacuum filtered through a series of filter screens. The residue from the final filter is washed and settled, has the supernatant removed, is centrifuged, again has the supernatant removed, and finally is placed upon a light transmission microscope for examination.

Every portion of the residue must be microscopically examined by an individual well acquainted with Giardia sp. A CDC report indicated 20 to

35 percent of public and private laboratories could not correctly identify intestinal protozoa [13]. Consequently, there is substantial room for error at this point.

In addition, there is room for error throughout the sampling and processing procedure. Cysts may be missed in sampling or they may be lost in each of the washing and filtering steps. Consequently, a finding of no cysts does not, in fact, mean the water is free of such organisms. Only positive samples say anything for sure, and even then nothing is known regarding viability or infectivity.

While the EPA filter technique has demonstrated advantages and has been improved recently [47], the procedure is not without its shortcomings. For example, the quantity of water required for filtration was originally established at 2,000 liters (approximately 520 gallons). This was based upon the following assumptions: one viable cyst is infective to humans; an individual drinks two liters of water per day; it would be desirable to detect one cyst per 20 liters (based upon research findings that a dose of 10 cysts had confirmed infectivity in humans); the efficiency of recovery of the method would be 1 percent [13]. Recent work, however, has led to recommendations changing the screen material used in processing and changing the desired sample volume to 100 gallons (i.e., due to interference from dirty water) [24]. The recent modifications to this apparatus have resulted in a more compact and transportable version which is also capable of sampling for viruses and bacteria [47].

A modification of the EPA method has been developed by David L. Rosgen of the Arapaho and Roosevelt National Forests (see Figure 4) [48]. This technique employs the EPA filter incorporated in a small flume which is placed in the river of interest. Discharge is captured at the outlet end

Figure 4. Forest Service Instream Giardia Sampler.

utilizing a graduated cylinder and a flexible hose (such as used for travel trailer holding tanks). This discharge is timed to establish a flow rate which is utilized in rating the particular installation. After a suitable time/discharge, the installation is again flow-rated (to establish an average discharge over the sampling interval), the sampler is removed, the sample filter is extracted and then transported on ice in a sealed bag to the laboratory for processing as described above.

The modification of the EPA technique has certain advantages over the existing method for application to the wildland setting, foremost of which is portability. This technique is backpackable and requires no pump or power source. In addition, it tends to smooth temporal variations to provide a more representative sample. To a limited degree, it can be flow proportional, but only to the extent that flow is confined, the sampler receives most of the flow and there is substantial grade. Another advantage lies in the slow discharge rates associated with the sampler. The device can be installed and left without monitoring for extended periods. Consequent to this is the advantage of large sample volume.

Both the EPA method and its modification offer potential for sampling Giardia. The appropriateness of the technique will be a function of the setting and objectives of the study. The methods, however, share a common shortcoming. Their efficiency and sensitivity are not known at this time. As a result, the detection of cysts reveals little information about the level of contamination [47,48].

6.0 Data Needs

The evaluation of Giardia sp. in the environment cannot be adequately completed until data are available on certain central questions. Among

these are: What are the spatial and temporal distributions of the organism? Is there a seasonality or discharge dependent relationship? To what degree is Giardia infection in man passed from wild animals? To what degree are wild animals infected by man? What are "normal" baseline or endemic levels of the organism? What are the mechanisms operative in cyst transport, and to what degree do they function? What timeframes are involved in transport and residence? What environmental extremes render cysts nonviable or noninfectious? How many species are there, and what is their host specificity? Other questions which need additional data relate to immunity mechanisms in humans, water treatment methodologies, etc. Such are beyond the scope of this presentation and shall not be addressed here.

Recent presentations by researchers and administrators indicate a significant divergence of opinion on a key issue, namely significance. Some researchers and administrators indicate substantial concern, i.e., EPA is considering the utilization of additional or changed treatment requirements for municipal water systems or a change in indicator organisms for efficient limitation standards (away from coliforms, etc., towards other microbiological indicator organisms) [10]. Other individuals emphasize the worldwide endemic nature of the organism, the lack of a life threatening hazard in the disease, a readily available treatment program, and a tendency for the disease to be self-limiting [25].

7.0 The Forest Service Role in Giardia Studies

There can be a need and a place for Forest Service involvement in Giardia studies. This can, however, only be determined after an in-depth evaluation of local situations.

The most important question which must first be addressed is, "how consequential is this organism, and its associated disease, to the locale in question?" This will be a function of several considerations of which the following may serve as examples: (1) What is the history of Giardiasis outbreaks in the area? (2) What has been the etiology of these outbreaks (i.e., contaminated municipal water systems, contaminated wells, backcountry untreated surface water infection, etc.)? (3) Are there municipal watersheds of concern located on Forest Service lands? (4) Do proposed management activities have the potential to affect the incidence of the disease (e.g. transplanting beaver, sewage sludge applications, etc.)? (5) Is the area of concern subject to periodic overtaxing of municipal water and sewage treatment systems (e.g. ski areas)? (6) Are there animal populations in critical watersheds which may serve as reservoirs for infection? (7) Has there been a noticeable increase in infections of backcountry recreationists or a decrease in the quality of backcountry sanitation? (8) Is water quality a principle land management objective? (9) What is the size of the "at risk population"?

Once it has been determined that Forest Service involvement might be desirable, the limitations of data collection should be clearly understood. These limitations include: the errors in sampling and sample processing; the inability to say definitively that a negative sample indicates the water sampled is free from Giardia; the inability to say a positive sample indicates likelihood of infection (due to lack of knowledge about viability, infectivity and host specificity); and the inability to clearly define the source of infections (i.e., animal, human, etc.).

With the foregoing in mind, there are also numerous logistical problems to be overcome, for example, a need for sample examination by

qualified personnel. Potential sources of help might include: local universities; State Departments of Health; Colorado State University, Wild Animal Disease Center; EPA; and U.S. Department of Health, Education and Welfare Center for Disease Control. It should be noted that initial sample preparation can be learned and accomplished by Forest Service personnel in their laboratories if certain pieces of equipment are available, thus reducing cost and impact to labs doing microscopic examinations.

The acquisition of samplers may be something of a problem. The sampler for the modified EPA method may be fabricated by a local metal shop due to inherent simplicity of design (see Figure 4). Information on this apparatus may be secured from the Arapaho and Roosevelt National Forests, Fort Collins, Colorado. The equipment for the EPA method is somewhat sophisticated and costly but may be used for viral and bacterial analyses as well. Information on its construction may be secured from the USEPA Health Effects Research Laboratory, Cincinnati, Ohio.

Another and perhaps more obvious problem involves manpower and funds. The EPA method requires more expensive equipment but less manpower. However, it must be used on water taps rather than natural streams. The modified method, while cheaper to begin with, is more demanding in its manpower requirements, especially if used in a backcountry setting.

In light of all the shortcomings and uncertainties described, what is to be gained in undertaking a Giardia study? For most Forests, it is likely that nothing will be gained. For some, however, management can be benefited. On those Forests, where a concern about Giardiasis exists, the data may serve an informational need (i.e., warning prospective forest users of high risk areas and preventative measures); it may help provide a

cooperative service (i.e., identifying high risk areas or times for downstream municipal water systems); it may help define a managerial objective (i.e., altering recreation use patterns, stream flow regimes or animal populations); it may facilitate a managerial service (i.e., providing water and/or sewage treatment or improving same), or it may help guide the development of a land use planning/controlling strategy (i.e., controlling housing development, locating sewage disposal facilities, making a sewage treatment effectiveness evaluation, etc.).

Such a study may provide information which will help fill some of the many existing information gaps. For example, the data can help to determine: (1) temporal and spatial distribution; (2) baseline or endemic levels; (3) point sources; (4) correlation with animal or human populations; (5) impact of land management on organism abundance; and (6) the correlation of water quality parameters to organism occurrence and/or abundance.

Furthermore, once the technical questions regarding viability, infectivity, host specificity, etc., have been adequately answered, it should be a relatively simple matter to correlate with previously acquired data to handle the more difficult and, perhaps, more fundamental questions germane to forest management.

8.0 Conclusion

Giardia sp. is an organism whose impact, as those who have experienced it can attest, can be debilitating and disconcerting. Although it is endemic throughout the world, it is either becoming more prevalent in the United States or more readily diagnosed. This has resulted in increased

awareness and concern, especially in Colorado and increasingly in other states noted for their pure water and pristine environment. As a consequence, there exists both an opportunity and a need, in certain situations, for wildland hydrologists to evaluate water quality from a microbiological perspective with a view towards providing assistance to both management and downstream water users.

Concomitant to this need is an imperative evaluation of the question of significance, a question which is difficult, at best, to answer when even the experts in the field disagree upon an appropriate answer.

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